
LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 5 – Introduction to Rodent Breeding Colony Management

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1. BEFORE YOU START BREEDING RODENTS

1.1. Before setting up a new breeding colony, consider the following:

- **Are the animals available from commercial sources? If yes, why breed them in-house?**
Although it may seem easier to maintain rodent strains in your own facility, the costs of doing so should be considered. It may be more cost-effective to order experimental animals as needed, rather than maintaining a breeding colony.
Approved commercial suppliers ensure genetic quality and are regularly evaluated by the veterinarians to meet specific quality standards. In addition, purchasing animals from a commercial supplier prevents the unnecessary culling of animals of the undesired sex or age and avoids overproduction of animals that cannot be used experimentally.
- **How much space can be allocated for this colony?**
We encourage you to communicate with the animal facility management to ensure that there is sufficient space to accommodate the colony.
- **Are trained personnel available to manage the colony?**
The most common reason for genetic variation is human error. Consider the time it will take to adequately manage a breeding colony, including record keeping. Typically, breeding cages should be monitored (ex: for births/weanings/breeder cage replacements) twice per week at a minimum.
Is there personnel available to cover absences?
- **Will both sexes, different ages and different genotypes (homozygotes vs. heterozygotes) will be used for experiments?**
Consider using both sexes (as phenotypes may vary between males and females), heterozygotes as controls, and wider age range to make the best use of the offspring.
- **What will be done with the offspring that cannot be used for experiments?**
Can they be used as controls, transferred to other protocols, donated to the animal facility for workshop purposes?

1.2. Determine crosses necessary to generate animals of interest

- Determine the genotype needed for colony maintenance, experimental animals, and controls. Consider whether mutations are recessive, dominant, semi-dominant mutations, if homozygous animals are viable.
- Consult vendor, supplier, or collaborator for added detailed information that can help to determine appropriate breeding scheme to use, and what to expect as of strain productivity, known health issues, phenotypes, etc.
- Employ proper strain nomenclature to describe your rodent models and use it consistently on cage cards, colony records, and animal use protocols. Nomenclature follows established rules and guidelines on Standardized Genetic Nomenclature. For example, C57Bl/6J is not the same as C57Bl/6N. These are completely different strains. <http://www.informatics.jax.org/mgihome/nomen/index.shtml>.
- Breeder genotypes should be verified before setting up matings.



- Examples of simple breeding schemes (crosses):
 - Homozygous mutant (-/-) x homozygous mutant (-/-) :
 - Can be used if both genders are viable and fertile as homozygotes
 - Offspring: 100% homozygous; genotyping not required
 -

Homozygous cross for mutant KO (-/-)		Male Genotype		
		-	-	
Female	-	-/-	-/-	Offspring outcome
	-	-/-	-/-	

- Heterozygous mutant (-/+) x homozygous mutant (-/-) :
 - Can be used if only one sex is viable and fertile as a homozygote (the other gender may be infertile or have reduced fertility, embryonic lethal, or die before reaching sexual maturity).
 - Even if they can be recognized by a visible phenotype, e.g., fluorescent proteins, coat color, etc., all mutant animals should be genotyped to differentiate homozygotes and heterozygotes.
 - Offspring: 50% homozygous; 50% heterozygous; genotyping needed.

Heterozygous (+/-) x Homozygous (-/-) cross for mutant KO		Male Genotype		Offspring outcome
		-	-	
Female	+	+/-	+/-	50% +/- 50% -/-
	-	-/-	-/-	

- Heterozygous mutant (-/+) x heterozygous mutant (-/+):
 - Can be used if neither gender is viable or fertile as homozygotes, e.g., homozygous mutant animals are severely impaired, infertile, embryonic lethal, or die before reaching sexual maturity.
 - If the mutant homozygotes and heterozygotes cannot be visually distinguished, individuals must be genotyped.
 - Offspring: 25% homozygous; 50% heterozygous; 25% wildtype; genotyping probably required

Heterozygous (+/-) x Heterozygous (+/-) cross for mutant KO		Male Genotype 		Offspring outcome
		+	-	
Female Genotype 	+	+/+	+/-	25% +/+
	-	+/-	-/-	50% +/- 25% -/-

- Complex breeding schemes (crosses):
 - Hemizygous mutants (such as Cre strains): Cre reporter alleles need to be present only in one copy for expression of the gene. When crossed with a strain containing a homozygous floxed gene, the Cre expression will delete the segment between floxed sites. Be aware that Cre hemizygotes crossed with Cre hemizygotes is often deleterious and should be avoided unless otherwise stated by recognized sources.
 - Hybrid strains with two or more mutant alleles: some strains must be produced by crossing animals from two strains. Thus, three colonies should be maintained: one for each of the parent strains, and one of the desired F1 strain.
- Outbred Stocks: To maintain genetic diversity in an outbred colony, matings between related individuals should be avoided. Keep the colony at a minimum size of approximately 25 breeder pairs per generation.

Examples of Inbred and Outbred Strains

	Inbred Strains	Outbred Strains
Mouse	C57Bl/6 Balb/C C3H DBA/2 FVB/N AJ	CD-1 Swiss Webster ICR SKH1
Rat	Lewis Fisher Brown Norway	Wistar Sprague Dawley Long Evans

1.3. Calculating the number of animals to produce

- It is important to manage your animal colonies to produce only the animals needed to maintain the colony and to generate the appropriate experimental cohorts. The CCAC guidelines require that all animals must be accounted and justified. Therefore, you are required to account for all animals generated to maintain the line, the experimental animals, and all controls. It is not ethically responsible to produce animals without a specific experimental purpose.
- To determine the number of experimental animals needed, consider the following:
 - What genotypes are needed for your experiments. Calculate and estimate the percentage of correct offspring that will be born, based on the proposed mating scheme
 - The acceptable age range that can be used
 - Which sex will be used
 - How often you need the animals
 - Breeding scheme
- To determine the number of matings needed to produce your experimental animals, consider what breeding scheme is needed based on the breeding colony's productivity. Important points to consider:
 - Average number of pups per litter based on strain and genotype
 - Average number of litters produced per breeding female
 - Breeding lifespan of your mice
- Use tools such as the Jackson's Laboratory Mouse Breeding Colony Size Planning Worksheet ([Annex 1](#)).
- Establish a timeline for the production of experimental animals.

1.4. Starting a new breeding colony

- How many breeding pairs should I obtain to start my own colony?
 - A minimum of 2-4 breeding pairs are recommended for most strains. Additional pairs are suggested for strains that can be challenging to breed, or to expedite colony expansion.
 - Consult the vendor or collaborator for general information on breeding performance.

1.5. Maintaining a breeding colony

- How many breeding pairs should I keep to maintain my colony?
 - A minimum of 6 breeding monogamous pairs or 3 breeding trios representing different generations in your colony is recommended.
 - Retain two generations of a strain, and do not eliminate one until the next one is producing pups. This can mean that there will be a period of overlap where you will be maintaining three generations at the same time.
 - Consider cryopreserving a strain that is not being actively used to generate experimental animals or in case breeding performance either declines, ceases, or a catastrophic event (such as a fire or pandemic) threatens your colony.

2. REPRODUCTIVE BIOLOGY

	SEXUAL MATURITY	REPRODUCTIVE LIFESPAN	LENGTH OF GESTATION	PUPS PER LITTER	AGE AT WEANING
Mouse	6-8 weeks Females as early as 5 weeks	7-12 months	19-21 days	2 to 12+ 6-8 on average Strain dependent Decreases with age	21-28 days
Rat	10-12 weeks Females as early as 8 weeks	12-15 months	21-23 days	4 to 20+ 8-10 on average Strain dependent Decreases with age	21-23 days

2.1. Mice

- Mice are polyestrous and breed year round; ovulation is spontaneous.
- The duration of the estrous cycle is 4–5 days. The cycle is divided into four characteristic phases: proestrus, estrus, metestrus and diestrus. The stage of the estrous cycle can be determined by vaginal cytology.
- Pregnancy may be confirmed by gentle abdominal palpation as early as gestation day 10. Weight gain of more than 2g between Day 8 and 10 can also be a good indicator of gestation.
- Mice can have consecutive litters due to a fertile postpartum estrus, i.e., one litter every 21 days if male breeder is kept with the female. The postpartum estrus occurs between 14 and 24 hours following parturition. When the postpartum estrus is missed, females will not be receptive to mating again until after the litter is weaned.
- Simultaneous lactation and gestation may slightly prolong gestation.
- Litter size and frequency of litters will decrease with age.
- The total number of litters per female will differ across strains and individuals (see table).

2.2. Rats

- Rats are polyestrous and breed year round; ovulation is spontaneous. Mating is usually nocturnal.
- The duration of the estrous cycle is 4–5 days. The cycle is divided into four characteristic phases: proestrus, estrus, metestrus and diestrus. The stage of the estrous cycle can be determined by vaginal cytology.
- Pregnancy may be confirmed by gentle abdominal palpation as early as gestation day 10.
- A fertile postpartum estrus occurs within 48 hours of giving birth and matings at that time are at least 50% successful. Failure to conceive during the postpartum estrus will delay breeding until two to four days after the litter is weaned.

3. SETTING UP MATINGS

3.1. Tips to optimize breeding performance

- Select healthy animals as breeders.
- Pairing young females with older males can improve breeding performance.
- Provide sufficient nesting material and adequate environmental enrichment in the cages.
- Handle breeding cages gently and place in a low-traffic area of the housing room.
- Avoid handling cages with newborn litters. The most critical days are within the first 7 days after birth.

- Observe breeder cages frequently, twice per week at a minimum.
- If breeding difficulties are noted in the colony, consult with a veterinarian as soon as possible, as fertility decreases with age.

3.2. Breeding schemes

- Monogamous pair
 - One male and one female are housed in the same cage for mating.
 - The mice are not separated when the dam becomes pregnant or delivers the pups.
 - Takes advantage of postpartum estrus and allows the female to become pregnant and nurse at the same time.
 - Litters are born approximately 21 days apart.
 - The 3-week-old litter must be weaned prior to the birth of the new litter.
 - For strains that require pups to be weaned later than 21 days of age, female must be separated from the male to avoid postpartum estrus and overcrowding.
- Trio breeding
 - One male and two females are housed in the same cage for mating.
 - Only acceptable for strains with average litter sizes of 7 pups per litter or less.
 - Both lactating females may be left in the same cage (+/- the male) only if each female has a litter of 7 pups or less.
 - Pups must be weaned at 21 days of age, prior to the birth of new litters.
 - For strains that require pups to be weaned later than 21 days of age, both females must be separated from the male once pregnancy is confirmed to avoid postpartum estrus and overcrowding.
 - Trios maximize breeding efficiency because both dams will help care for the young, and are most appropriate for strains that generate small litters or are difficult to breed.
- Harem breeding
 - Due to increased risk of overcrowding and impact on animal welfare, harem breeding is only permitted under specific circumstances and must be justified in the Animal Use Protocol. One example when harem breeding is necessary is when embryos of a specific age will be used for tissue collection purposes.
 - One male and up to 4 females are housed together for mating.
 - Pregnant females must be separated into another cage before parturition to avoid overcrowding. No litters should be born into cages with harem breeding.
 - Does not utilize postpartum estrus.
 - In vitro fertilization (IVF) can be an alternative to generate a large cohort of animals from a single male more efficiently than harem mating.
- Male rotation
 - A single male is rotated through a cage with 1-2 females each week.
 - Once male is removed, do not return him to the cage of females until pups are weaned. Introducing a male into a cage with a female and her litter may lead to aggression.
 - Does not utilize postpartum estrus.

3.3. Replacing breeders:

- To maintain optimal breeding performance, breeders should be replaced before their reproductive performance begins to decline.

- Breeding success decreases when mice are older than 8 months old and when rats are older than 9 months old. It is generally recommended not to keep breeders over 1 year old.
- Do not replace all breeding animals at the same time. It is best to have breeding animals of various ages in the colony. Stagger the creation of new breeding cages, never replace all breeders at once.
- Plan to replace breeders on a regular basis, e.g., replace one breeding pair each month.
- Replace breeders if:
 - No litters have been born 60 days after mating or 60 days after weaning of the last litter and female is not pregnant (90 days for strains known to have low fertility).
 - Several litters have been born but no pups have been weaned.
 - A significant decrease in litter size is noted, e.g., 1-2 pups born per litter when previously average litter size was 8-9 pups.
 - A female has produced 6 litters.
 - Health issues develop.

4. GENETIC DRIFT

4.1. What is genetic drift?

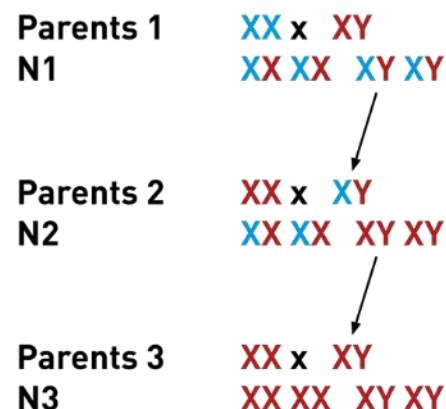
- Genetic drift refers to spontaneous changes in genomic DNA that can arise in any generation. Drift comes in the form of DNA replication or repair mistakes that get passed on in the germ cells.
- These mutations may appear as silent, unimportant fluctuations in the genetic makeup of an individual. However, these seemingly insignificant mutations can lead to a change in the phenotype of the strain and become the source of unexplainable experimental irreproducibility. Genetic drift may result in substantial genetic divergence between colonies of the same strain.
- Often drift does not produce a visible or detectable phenotype and may easily go unnoticed. Occasionally, drift may be detected through the observation of phenotypic differences such as:
 - Growth retardation or "runting"
 - Reduced breeding performance
 - Changes in appearance, such as coat color
 - Spontaneous clinical observations
 - Altered phenotypes and performance in experimental models
- True detection of genetic drift requires comparison of whole genome sequence data.

4.2. Preventing genetic drift?

- Although genetic drift is a spontaneous biological process that cannot be stopped, it's potential negative impacts can be minimized through the following practices:
 - Maintain detailed colony records.
 - Watch for phenotypic changes in mutants and controls.
 - Refresh breeders frequently, every 6-10 generations. This can be accomplished by simply purchasing new breeders from a vendor for lines that are commercially available or by backcrossing the line to the inbred background strain. See How to Refresh Your Mutant or Transgenic Mouse Strains, section 4.3.
 - Avoid selection pressure (choose breeders at random).
 - Cryopreserve unique strains that either were created in your lab, or that may be difficult to reacquire. Cryopreservation protects your mouse strains and your research programs from disasters such as genetic contamination and genetic drift. If a breeding error, genetic drift, loss of phenotype, or a pathogen outbreak hits your colony, you can recover the cryopreserved strain and continue your studies with minimal lost time.

4.3. How to refresh your mutant or transgenic strains

- Wildtype (WT) animals to be used for refreshing a strain should be purchased directly from a commercial vendor.
 1. Breed a homozygous female from the “drifted” colony (blue XX chromosomes) to an WT inbred male from a trusted vendor (red XY chromosomes), producing all heterozygous pups (N1).
 2. In the next generation, take an N1 heterozygous male (blue X, red Y) and mate with a WT inbred female from a trusted vendor (red XX) to generate N2 backcrossed mice.
 3. Repeat step 2: take an N2 heterozygous male (now red XY) and mate with a WT inbred female (red XX) to generate N3 backcrossed mice.
 4. Last, mate female and male N3 heterozygotes (all sex chromosomes are now red and “refreshed”) to re-homozygose the knockout allele (not shown).



5. WEANING LITTERS

5.1. What is weaning?

- Weaning, in a laboratory setting, refers to the day the litter is removed from the mother.
- It coincides with a period defined by a sharp decline in maternal investment and of offspring suckling, the commencement of offspring eating solid food, changes in offspring gut enzyme activity, and an increase in offspring socialization and exploration.

5.2. When to separate pups from the mother

- Rodents can be weaned between 18 and 28 days of age.
 - Mouse pups are generally weaned at 21 days of age. At weaning time, mice should weigh at least 10-12g and are fully capable of eating and drinking independently.
 - Rats are weaned between 21 and 23 days of age.
- Weaning at 21 days is mainly due to the fact that continuous mating strategies, i.e., leaving males with females continuously, are adopted to maximize the breeding success and productivity.
- Pups must always be weaned if the dam gives birth to a new litter. This would be the case if the mother becomes pregnant during the post-partum estrus, immediately after giving birth. Dams cannot simultaneously nurse two litters. Older litter may hijack milk and trample neonates.

5.3. Scheduling weaning

- Observe breeding cages regularly to record date of birth with as much accuracy as possible. Twice per week at a minimum.
- It is relatively simple to estimate the age of a litter from birth to 14 days by closely observing physical attributes. Refer to [Annex 2: Mouse Pup Appearance by Age](#).
- Some animal facilities will record date of birth and expected weaning date on cages with litters. However, this type of service cannot replace regular monitoring of the breeding colony.

5.4. When should pups be left longer with the mother?

- Pups of some strains may not develop at the expected rate and may benefit from being left longer with the mother. Very small, poorly developing pups are referred to as runts. The presence of runts may indicate a genetic abnormality, or competitive disadvantage within the litter.
- Avoid weaning underweight pups (mice weighing under 10g, rats weighing under 30g) or pups that are not in optimal health.
- Pups can be left with the mother for a maximum of 28 days.
- For strains where pups will be routinely weaned later than at 21 days of age, females must be separated from the male before giving birth to avoid postpartum estrus. Females cannot simultaneously nurse two litters. Older litter may hijack milk and trample neonates.
- Refer to [Annex 4](#).

5.5. How to separate weanlings

- Upon weaning, separate males and females by sex.
- Avoid weaning single animals. Consider pooling weanlings of similar ages from different litters when possible.
 - Females from different litters can be pooled into the same cage.
 - Males from different litters can only be pooled if weaned on the same day. Pairing males after weaning results in aggression that can be severe.

5.6. Weanling care

- For cages with automatic watering systems, the valve should be primed before placing the cage in the rack so that a drop of water is present on the valve. This will encourage the animals to drink from the valve.
- Food pellets can be provided at the bottom of the cage.

5.7. Small weanlings

- Some strains may not develop at the expected rate and may require additional care upon weaning, even if they have reached the minimum weight (10g for mice and 30g for rats) and/or maximum age for weaning. If assistance is required, contact your veterinary care service.
 - Small weanlings must be monitored daily.
 - Food pellets, dry or moistened, may be provided to weanlings at the bottom of the cage.
 - A water bottle can be provided, regardless of the standard watering system. For cages with automatic watering systems, the valve must always be available. The valve should be primed before placing the cage in the rack so that a drop of water is present on the valve as this will encourage the animals to drink from the valve.
 - Soft dough or gel diets are commercially available such as DietGel® or NutraGel Diet™.
- If small weanlings remain debilitated, e.g., hunched, dehydrated, lethargic, with rough coat even with supportive care, Veterinary Care staff should be notified.

5.8. Avoiding overcrowding

- Overcrowding refers to cages with more animals than allowed by federal and institutional guidelines.
- Overcrowding may cause discomfort and stress to animals, lead to increased mortality, and decrease colony productivity. As such, overcrowding can become a serious animal welfare concern.
- To avoid overcrowding, observe breeder cages regularly. Schedule times for weaning at least once weekly.

5.9. How many mice can I house in each cage?

- Consult your animal facility's housing density requirements as these may vary by cage type. See examples in [Annex 3](#).
- The typical acceptable cage density for mice is as follows:
 - 5 adults: all males or all females
 - 1 breeding female +/- 1 breeding male + 1 litter
 - 2 breeding females +/- 1 breeding male + 2 litters only if strain typically produces litters of ~~8~~7 pups or less. Pups must be removed when they reach 21 days of age.

5.10. How many rats can I house in each cage?

- Cage density for rats is dependent on the cage type and weight of the animals.

6. IDENTIFICATION METHODS

6.1. Selecting an identification method

- The selection of identification methods should take into consideration:
 - Whether the animals will be single or group housed
 - The age of the animals at the time of identification
 - Whether the method needs to be temporary or permanent
 - Whether the collection of tissue for genotyping is required
 - The least invasive identification method available should be selected

6.2. Cage cards

- Use cage cards to identify individually-housed mice or a single breeding pair.
- Use cage cards to identify groups of rodents on protocols where individual identification is not necessary.
- Cage cards must include, at a minimum, the following information:
 - Principal Investigator
 - Protocol number
 - Species
 - Strain
 - Sex
 - Number of animals in the cage

6.3. Temporary markings:

- Temporary marking can be used for short-term individual identification.
- Use a non-toxic, indelible marker to write numbers, bars, or other distinguishable markings, on the tail or the ears.
- If temporary marking is to be used for a duration exceeding a week, repeat markings every 2-3 days.

6.4. Ear punching

- Rapid and minimally invasive method that also yields a tissue sample for genotyping.
- Does not require anesthesia and analgesia.

- This method should not be used in rodents under 2 weeks of age.
- Procedure:
 - Ensure the ear punch apparatus or scissors are sharp.
 - Disinfect the ear punch or scissors with 70% alcohol and wipe dry before use.
 - Restrain the animal securely by the scruff.
 - Using the ear punch, punch holes and/or notches in the ears, following an identification chart.
 - Whenever possible, use a simple code to limit the number of notches/punches. Alternatively, use scissors to make small notches in the ears. See sample, [Annex 5](#).
 - Have the identification chart readily available in the animal room to allow prompt identification of individuals.
 - Use the excised tissue as a sample for genotyping. Place in well-identified collection tube.
 - Disinfect ear punch or scissors with 70% alcohol between animals.

6.5. Ear tags:

- Provide a unique identification number for each animal.
- Does not require anesthesia and analgesia
- This method should not be used in rodents under 2 weeks of age.
- Proper care in placing ear tag is important and improperly placed tags can result in a loss of a tag, inflammation, excessive scratching which may cause injury.
- This method can be combined with ear punching on the opposite ear for tissue collection for genotyping or to avoid issues that arise if two or more animals lose an ear tag in the same cage.
- Procedure:
 - Use tags that are about 5 mm long.
 - Rinse the tags in 70% alcohol before use.
 - Place the tag low on the pinna (distal 1/3) so that it rests against the animal and does not bend the ear, cause the animal to hold its head in a lopsided manner, or catch on the cage.
 - Monitor site of implantation for local infection or inflammation.

6.6. Micro-tattooing:

- Use a micro-tattooer to inject tattoo ink in the toe pads or the ears or the tail.
- This method is suitable for both neonates and adults.
- Whenever possible, use a simple code to limit the number of toes tattooed.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals. See sample, [Annex 5](#).

6.7. Microchips:

- Do not implant microchips in animals less than 3 weeks old.
- Implant microchips subcutaneously according to the manufacturer's recommendations.
- A compatible reader is required to allow identification of the mice.
- This method requires the use of general anesthesia and analgesia. Refer to corresponding SOPs.
- Procedure:
 - Clean the site of microchip implantation with skin disinfectant, e.g., 2% chlorhexidine solution.
 - Using the implanter, inject the microchip subcutaneously in the neck area.

- Reuse microchips only after proper cleaning and sterilization (follow manufacturer's recommendation).

6.8. Tattooing:

- This method requires the use of general anesthesia and analgesia. Refer to corresponding SOPs.
- Use an electric tattoo machine to write numbers on the tail.
- Ensure that needles are sterile and sharp.

6.9. Distal phalanx biopsy (toe amputation)

- This method is not acceptable when used for the sole purpose of identifying rodents.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals. See sample, [Annex 5](#).
- Refer to section 7.8

7. COLLECTING TISSUE FOR GENOTYPING

To maximize the efficiency of the breeding colony, genotyping samples should be processed in a timely manner and animals of the undesired genotype removed quickly.

7.1. Selecting a tissue collection method

- The selection of the tissue collection method should take into consideration:
 - The age of the animals at the time of tissue collection
 - The least invasive method available should be selected

7.2. Fecal pellet

- This method is minimally invasive and may be useful with rodent strains prone to bleeding disorders.
- Procedure:
 - Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding for a few minutes.
 - Identify animal as per Rodent Identification SOP.
 - Place fecal pellet in an identified collection tube.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

7.3. Skin swabbing

- This method is minimally invasive and may be useful with rodent strains prone to bleeding disorders.
- Procedure:
 - Restrain the animal.
 - Using a cotton-tipped swab, stroke the ventral and dorsal skin against the direction of hair growth.
 - Perform a minimum of 3 strokes of 3cm in length each.
 - Insert cotton bud into collection tube and snip off excess shaft.
 - Identify animal as per Rodent Identification SOP.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

7.4. Buccal epithelial cell

- This method is minimally invasive and may be useful with rodent strains prone to bleeding disorders.
- Procedure:
 - Firmly restrain the animal by the scruff to maintain its mouth open.
 - Using a cotton-tipped swab with a <2mm bud, vigorously rub the inner cheeks while rotating the swab, avoiding the tongue.
 - Insert cotton bud into collection tube and snip off excess shaft.
 - Identify animal as per Rodent Identification SOP.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

7.5. Ear punching

- Refer to section 6.4

7.6. Tail biopsy

- Tail snipping should be performed on mice between 14 and 21 days of age (ideally between 14 and 17 days).
- Tail biopsy can only be performed twice over the life time of the animal and cannot exceed 5mm total.
- Identify animal as per Rodent Identification SOP.
- Tail snipping procedure for mice less than 21 days of age:
 - General anesthesia is recommended but not required.
 - Gently, but securely, restrain mouse (manual or mechanical).
 - Swab the tail with antiseptic (e.g. alcohol).
 - Snip 2-3mm off the tip of the tail with sharp, sanitized scissors or disposable scalpel.
 - Remove biologic material and sanitize the scissors after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if you are snipping several mice tails.
 - Place tissue sample into an identified collection tube.
 - Check for bleeding before returning mouse to its cage. If bleeding occurs, do one of the following:
 - Apply a drop of tissue glue to tip of tail.
 - Apply a chemical cautery agent (e.g. Kwik Stop® powder or silver nitrate stick).
 - Electric or heat cauterize the cut end of the tail
- Tail snipping procedure for mice over 21 days of age:
 - Requires general anesthesia and analgesia.
 - Brief general anesthesia is provided with isoflurane, by placing animals in an induction chamber to achieve unconsciousness. Refer to Rodent Anesthesia SOPs.
 - Analgesia must be provided for 72 hours as per Rodent Analgesia SOPs.
 - Perform the tail snipping as described above.

7.7. Whole blood

- Collect as per SOP 403-Guidelines Blood Collection Volumes and Frequency.
- Note that this method may or may not provide sufficient DNA. Protocols for extraction of DNA may need to be adapted for the method.

7.8. Distal phalanx biopsy (toe amputation)

- This method is acceptable in circumstances where the genotype needs to be known before weaning. This method replaces the tail biopsy as a sample for genotyping and ear notching as an identification method.
- Animals must not be older than 7 days old at the time of the biopsy.
- No more than 2 digits (total) can be affected, and only 1 biopsy per paw.
- Following toe amputation, no further phalanx biopsy can be performed.
- Procedure:
 - Ensure the scissors are sharp.
 - Disinfect the scissors with 70% alcohol and wipe dry before use.
 - Restrain the animal securely.
 - Remove only the most distal phalanx, following an identification chart.
 - Use the excised tissue as a sample for genotyping. Place in well-identified collection tube.
 - Disinfect scissors between animals.

8. BREEDING PERFORMANCE

8.1. Factors influencing breeding performance:

- **Strain**

Genetic mutations may affect phenotype, including breeding performance. Some induced mutations are embryonic lethal, some cause infertility or reduced fertility or affect a females ability to produce insufficient milk for the pups.

- **Age**

Rodents have a relatively short reproductive span. Older animals are not optimal breeders.

- **Environment**

Breeding rodents require a quiet, comfortable, stable environment, free from noise and vibration, with a consistent light cycle.

- **Health status**

Do animals appear healthy?

- **Stress**

Noise, vibrations, over-handling, environmental enrichment

8.2. What is considered poor breeding performance

- Some strains breed poorly. It is important to know the breeding characteristics (fertility, fecundity, litter size) of your strain or related background strain so you know what to expect. Refer to background strain reproductive characteristics available from commercial vendors.
- Breeding performance of common inbred mouse strains:

STRAIN	PRODUCTIVE MATINGS	LITTER AVERAGE SIZE	AVERAGE # LITTERS
129/SvJ	75%	5.9	4.1
A/J	65%	6.3	2.9
Balb/CJ	47%	5.2	3.8
C3H/HeJ	86%	5.7	2.9
C3H/HeOuj	99%	6.4	3.7
C57Bl/6J	84%	7.0	4.0
C57Bl/10SnJ	67%	6.3	2.8
FVB	>90%	9.5	4.8
SJL/J	72%	6.0	3.1

- Breeding performance of common rat strains:

STRAIN	LITTER AVERAGE SIZE
Sprague-Dawley	10
Wistar	10
Fisher 344	6
Lewis	7
Long Evans	11

- Accurate and assiduous recordkeeping will help to rapidly identify a decrease in breeding performance.
- Reproductive performance is among the characteristics most affected by inbreeding. Outbred animals and F1 hybrids of all types will routinely surpass the inbred strains.
- Poor breeding performance can manifest in several ways:
 - No litters being born in over 60 days
 - Decrease in the number of pups born per litter or increased neonate mortality
 - Weanlings too small at time of weaning or mortality before weaning age
- Contact the veterinary care staff of your facility as soon as you suspect that the reproductive performance of your strain is decreasing. Waiting too long can risk losing your line.

8.3. Factors responsible for poor breeding performance (see [Annex 6](#))

- Females do not get pregnant
 - Inability or lack of the physical act of mating
 - Shortened reproductive life or life span
 - Inability to fertilize
 - Inability to conceive
- Females become pregnant but don't give birth
 - Inability to sustain pregnancy
 - Embryonic lethality
- Females give birth but the pups do not survive
 - Absence or reduction in nursing capacity
- Only a small number of pups reach weaning age
 - Pups unable to suckle or have underlying conditions affecting their overall health

8.4. Common causes of poor breeding performance

- Breeders improperly sexed
- Mating started early or too late
- Unhealthy animals used as breeders
- Breeders not replaced at the optimal time – animals too old or have been in mating for too long, i.e., have previously produced too many litters.

- All breeders the same age, need to be replaced all at once
- In cage where the male is left with the female, pups are not weaned when new litter is born resulting in pup mortality.
- Female mortality due to complications giving birth (dystocia)
- Pups weaned too early or when they are too small leading to mortality
- Cages not monitored frequently enough leading to inaccurate recordkeeping
- Genetic drift
- Genetic mutations may affect phenotype, including a decrease in breeding performance.

8.5. Assisted reproductive technologies (ART)

- For issues related to conception/gestation, assisted reproductive techniques (ART) may be an option.
- Techniques commonly used include:
 - Exogenous hormone regimens for superovulation
 - In vitro fertilization
 - Embryo transfer
 - Ovarian transplantation

9. MONITORING AND RECORDKEEPING

9.1. Monitoring a rodent breeding colony

- Regular monitoring of rodent breeding colonies is essential.
- Monitor of a breeding colony should occur at least twice per week. Arrangements should be made to cover for absences.
- Observe and note the following:
 - General health of the colony animals
 - Breeding performance: births, litter sizes, etc.
 - Phenotypes
 - Plan weaning of litters
 - Plan replacement of breeders

9.2. Why is recordkeeping important?

- The data collected can be used to optimize breeding and improve the productivity of the colony.
- It allows you to detect a decrease in breeding performance early on.
- It facilitates the task for someone taking over the colony.
- It improves animal welfare as it significantly reduces the incidence of overcrowding.
- Data collected can be used to justify the number of animals required for the following year at the time of the Animal Use Protocol renewal.

9.3. Information to record

- All individual rodents in a breeding colony should be identified by one of the methods detailed in section 6.
- Different color cage cards or labels can be used to make each colony visually distinguishable.

- A centralized record keeping system should be used to keep track of mating pair set-ups and breeding outcomes. Various types of colony management systems exist including commercial products, free databases and templates, or you can create your own.
 - <https://www.softmouse.net>
 - <https://www.transnetyx.com/colony>
 - <https://www.future-science.com/doi/full/10.2144/04371BM05>
- Breeding colonies should be monitored regularly, approximately twice per week, to keep records accurate.
- Records should be reviewed regularly to detect possible decreases in breeding performance and to constantly evaluate the production of animals.
- To assess reproductive performance for a breeding cage, evaluate:
 - Time between mating and first litter
 - Interval between litters
 - Cumulative number of litters and/or offspring
 - Average litter size (birth vs. weaned).
 - Where applicable, comparison of offspring's genotype versus what would be expected based on parental genotypes and expected mendelian ratio.

9.4. Breeder Information

- Mating units can be assigned an individual number or record
- Cages of breeders should be clearly identified and cage cards should contain the following:
 - Identification, date of birth, and genotype (if applicable) of breeders
 - Strain name, using same nomenclature as specified in your animal use protocol
 - Mating date
 - Date of birth for all litters born, including those that do not survive
- In order to comply with CCAC guidelines, centralized breeding records should include:
 - Individual animal identification and parentage
 - Matings between animals
 - Date of breeding
 - Date of birth for all litters born, including those that do not survive
 - Litter size at birth
 - Number of mice that have been weaned
 - Gender frequencies
 - Interval between litters
 - Genotyping results of offspring, where applicable
 - Generation number
 - Individuals within litters that are used in experiments or to set up the next generation of matings
 - Expected and demonstrated phenotypes (including behaviors)
 - Number of animals euthanized including criteria used for culling animals.

10. REFERENCES

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GLOSSARY

Allele	An alternate form of a gene or locus.
Dam	The female parent of an animal
Generation	All of the offspring that are at the same stage of descent from a common ancestor
Genetic drift	The constant tendency of genes to evolve, even in the absence of selective forces. It is fueled by spontaneous mutations.
Genetic Mutation	The DNA gene is damaged or changed (introduction, modification, or removal) in such a way as to alter the genetic message carried by that gene.
Genotype	The genetic makeup or genetic description of an animal (Homozygous, heterozygous, hemizygous).
Hemizygote	Possessing an unpaired allele at a particular locus.
Heterozygous	Possessing two distinguishable alleles at a particular locus.
Homozygous	Possessing two identical alleles at a particular locus.
Hybrid Strain	Hybrid mice are made by crossing mice of two inbred strains together. The resulting F1 hybrids are genetically identical because at each gene they all carry both alleles from the two inbred parents.
Inbreeding	A strain that has been maintained by sibling (sister x brother) matings for 20 or more consecutive generations. <i>Examples of inbred mice:</i> C57Bl/6, Balb/C, C3H, DBA/2, FVB/N, AJ, AKR, <i>Examples of inbred rats:</i> Lewis, Fisher, Brown Norway
Locus	Any genomic site.
Offspring	The organism or organisms resulting from sexual reproduction.
Outbreeding	A cross between genetically unrelated mice. <i>Examples of outbred mice:</i> CD-1, Swiss Webster, ICR, SKH1 <i>Examples of outbred rats:</i> Wistar, Sprague Dawley, Long Evans
Parturition	The act or process of giving birth to one or more offspring.
Phenotype	The physical manifestation of a genotype
Polyestrous	Polyestrous animals exhibit more than one estrous cycle within a certain season, until they become pregnant or until the anestrous period begins.
Transgene	A fragment of foreign DNA (DNA construct) that has been incorporated into the genome of a mouse.
Transgenic	A mouse with one or more transgenes.
Weaning	The process of an animal ceasing to be dependent on the mother for nourishment.
Wild type (WT)	Refers to the phenotype of the typical form of a species as it occurs in nature. Originally, the wild type was conceptualized as a product of the standard "normal" allele at a locus, in contrast to that produced by a non-standard, "mutant" allele.

BREEDING COLONY SIZE PLANNING WORK SHEET

Determine your Research Needs

Line 1....How many mice do you need?

Line 2....What age range is acceptable for your experiments?

If they all must be born in the same week, enter 1

If age range is 2 weeks, (e.g., 5-6 weeks of age), enter 2

If age range is 4 weeks (e.g., 5-8 weeks of age), enter 4.....

Line 3....How often do you need the mice?

If needed weekly, enter 1

If needed every other week, enter 2

If needed once a month, enter 4.....

Line 4 ... Divide Line 1 by the smaller of Line 2 or Line 3

(round *up* to the nearest whole number).....

Line 5....What gender do you need?

If only one gender is needed (i.e. either male or female), enter 2

If both genders can be used, enter 1.....

Line 6....What breeding scheme are you using to maintain the colony?

If homozygote x homozygote, enter 1

If heterozygote x homozygote, (or the reciprocal) enter 2

If heterozygote x heterozygote, enter 4.....

Line 7....Can you do your experiment with fewer mice?

If yes, enter 1

If no, enter a "fudge factor" to ensure sufficient production of the mice
you will need (e.g., if you need 10% over, enter 1.1).....

Calculate the Number of Mice you Need to Produce Weekly

Line 8....Multiply the following: Line 4 x Line 5 x Line 6 x Line 7

(round *up* to the nearest whole number).....

Determine your Breeding Colony Productivity

Line 9....What is the average number of pups weaned per litter?

Line 10....How many litters are produced by each breeding female? (hint: a female will usually produce a litter ~every 2 months, if left with her mate continuously).....

Line 11....What is the breeding lifespan of your matings (in weeks)?

Calculate the Number of Weaned Pups per Female Each Week

Line 12....Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth).....

Calculate the Number of Breeding Females Needed

Line 13....Divide Line 8 by Line 12 (round *up* to the nearest whole number).....

Refining your Breeding Colony Size:

To ensure a consistent inventory of weaned mice, remove non-productive breeders (i.e. no pregnancy and no weaned pups by 60-90 days after mating or successfully weaning a litter) and/or breeders at the end of their breeding cycle:

- Replace equal numbers of mice weekly or monthly
- Raise enough mice to produce breeders as well as meet your experimental needs

Calculate Number of Breeding Females Needed to Maintain Colony

Line 14....To determine the number of replacement female breeders needed weekly, divide Line 13 by Line 11 (round *up* to the nearest half).....

Line 15....To determine number of additional females needed as breeder replacements, multiply line 14 by 2 then divide by line 12 (round *up* to the nearest whole number).....








































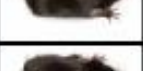





Line 16.....**Final Number of Breeding Females needed to maintain colony and provide sufficient mice for experiments, add Line 13 and Line 15.**.....

Note: There are situations in which this worksheet is less accurate, such as colonies maintaining sub-lethal genes or stocks with gene penetrance issues.

JAX® Mice Pup Appearance by Age

The approximate age of mouse pups can be determined by their physical attributes during the first two weeks of life.

Examples of the developmental stages of albino, agouti, and black pups are shown.

		C57BL/6J	C3H/HeJ	BALB/cJ
		000664 black	000659 agouti	000651 albino
Days of Age	0			
	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
	11			
	12			
	13			
	14			

How many animals in a standard mouse cage? Combien d'animaux par cage à souris standard?



5 adults
5 adultes

OR
OU



OR
OU



* pups must be removed when they reach 21 days of age
* les souriceaux de 21 jours doivent être séparés

OR
OU



Up to 8 juveniles (3-6 weeks of age)
Jusqu'à 8 juvéniles (âgés de 3-6 semaines)



Your Veterinarian
Votre Vétérinaire

How many animals in a standard rat cage?
Combien d'animaux par cage à rat standard?



4 adults < 200g each
4 adultes < 200g chaque

OR
OU



3 adults < 300g each
3 adultes < 300g chaque

OR
OU



2 adults > 300g each
2 adultes > 300g chaque

OR
OU



♀
With litter
Avec portée



Your Veterinarian
Votre Vétérinaire

How many animals in a ventilated double-decker rat cage?
Combien d'animaux par cage à rat ventilée double?



8 rats < 200g each
8 rats < 200g chaque

OR
OU



6 adults < 300g each
6 adultes < 300g chaque

OR
OU



4 adults > 300g each
4 adultes > 300g chaque

OR
OU

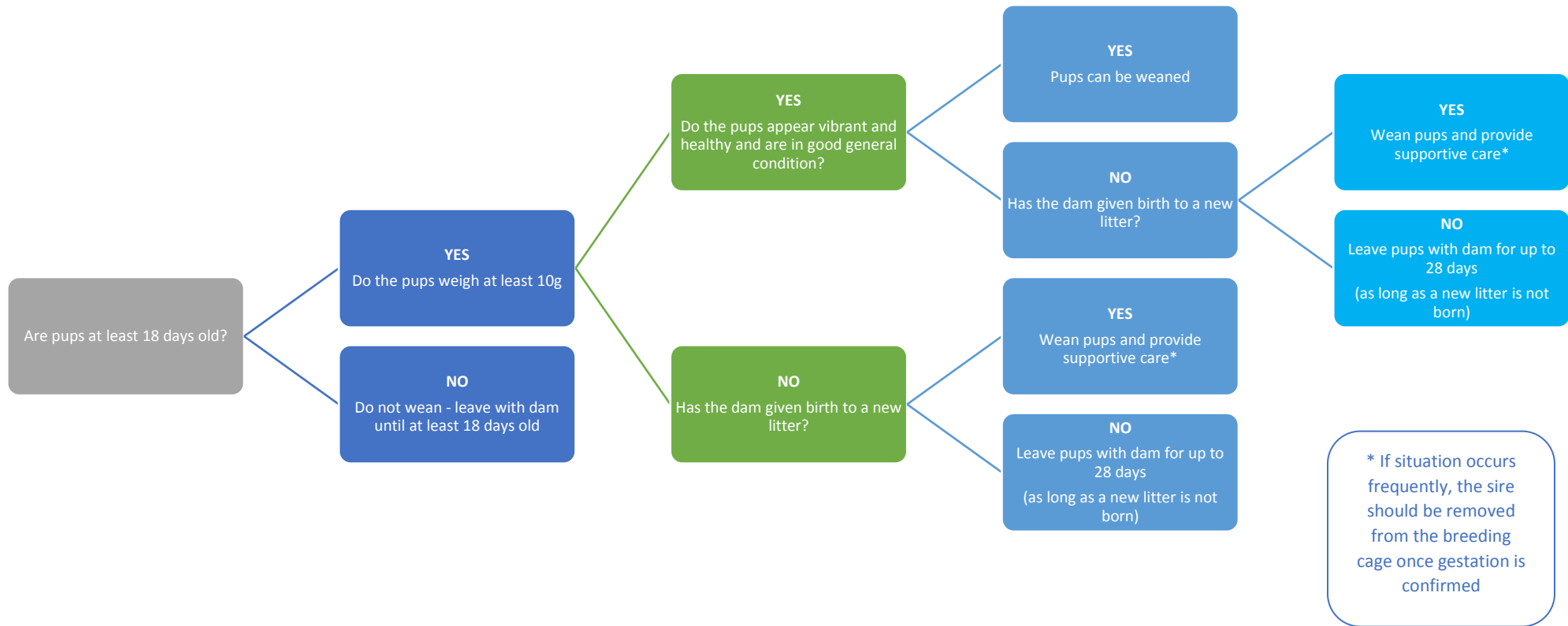


2 females with litter
2 femelles avec portée



Your Veterinarian
Votre Vétérinaire

When do I wean a litter?

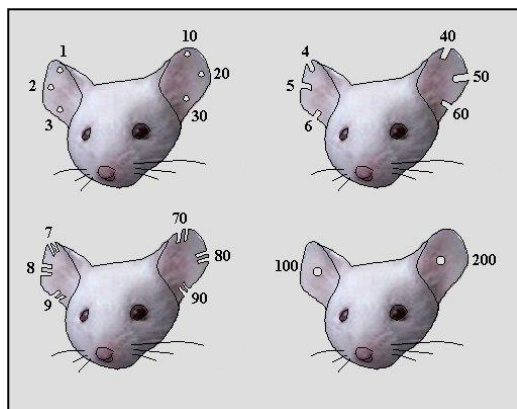


SUPPORTIVE CARE FOR SMALL WEANLINGS

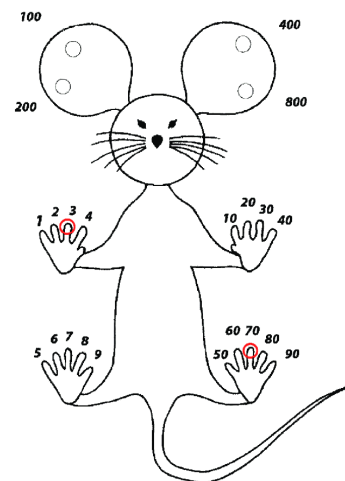
PROVIDE FOR 7 DAYS

- Food pellets, dry or moistened, at the bottom of the cage.
- A water bottle, regardless of the standard watering system. For cages with automatic watering systems, the valve must always be available. Prime valve before placing the cage in the rack so that a drop of water is present on the valve; this will encourage the animals to drink from the valve.
- If desired, provide commercially available soft dough or gel diets are such as HydroGel™, DietGel®, NutraGel Diet™.
- Monitor small weanlings daily until they are thriving.
- If small weanlings remain debilitated, e.g., hunched, dehydrated, lethargic, with rough coat, after 48 hours of supportive care, notify Veterinary Care staff promptly.

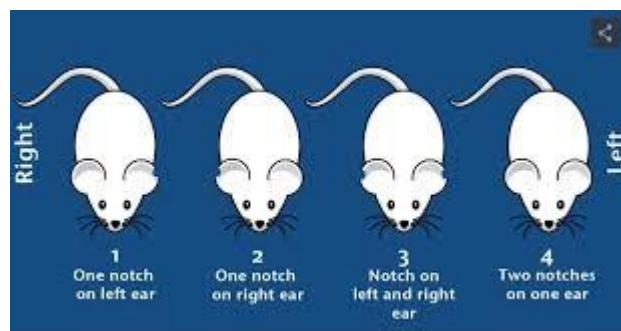
Ear notch code samples



Toe microtattoo or toe amputation code sample



Ear tag placements



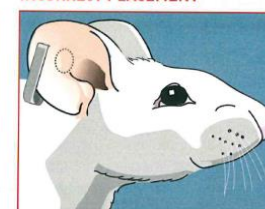
EAR TAG TARGET AREA



PROPER PLACEMENT



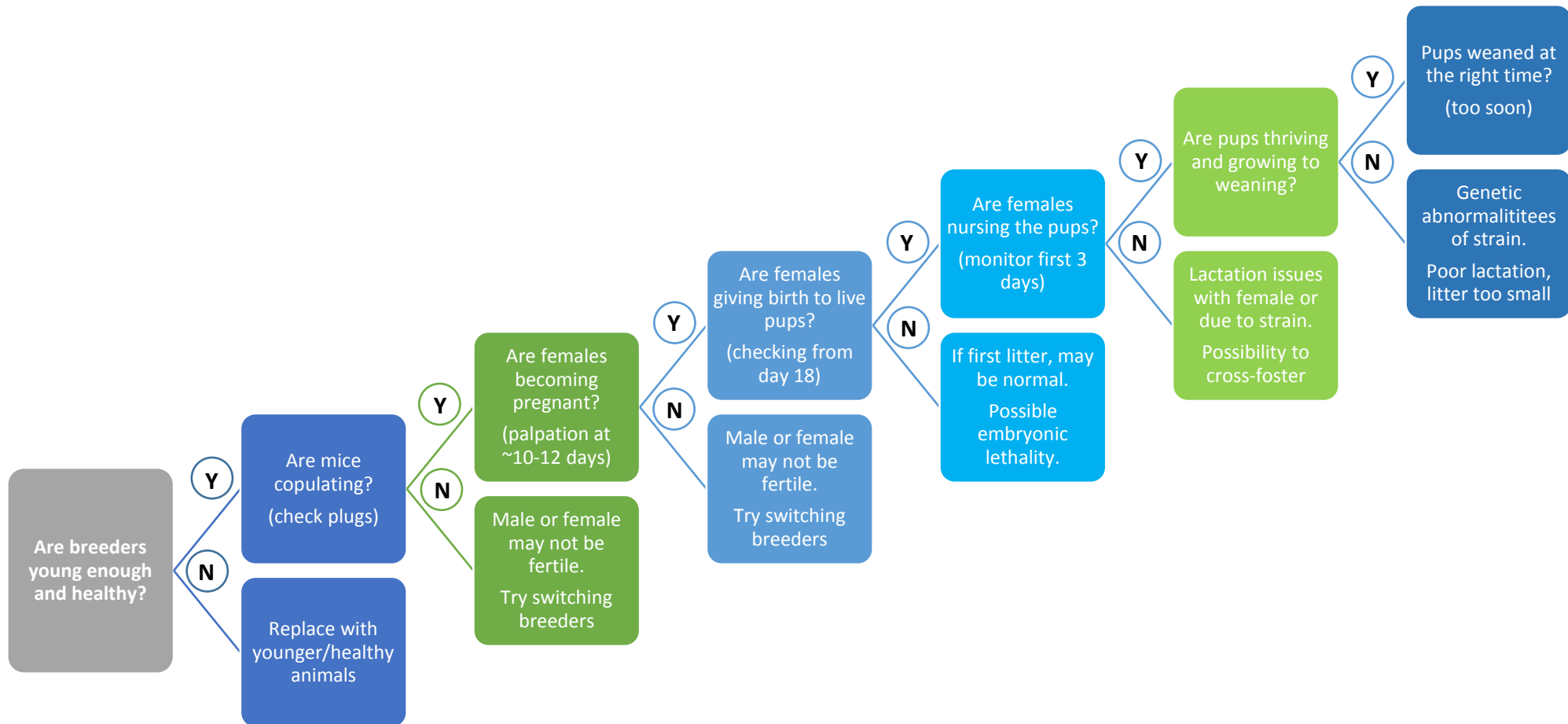
INCORRECT PLACEMENT



Position applicator such that the pointed end of the ear tag is within the area enclosed by the dashed oval and the curved end of the tag will be on the lower end of the ear. Do not insert the tag through the folds of cartilage that protect the auditor canal (shown as gray area).

The Jackson Laboratory

Determining the cause of poor breeding performance?



Contact the veterinary care staff of your facility as soon as you suspect that the reproductive performance of your strain is decreasing.

Waiting too long can risk losing your line.
